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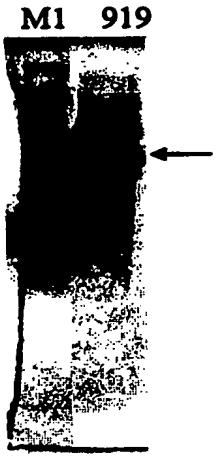
(54) Title: NEISSERIA MENINGITIDIS ANTIGENS AND COMPOSITIONS

(57) Abstract

The invention provides proteins from Neisseria meningitidis, including the amino acid sequences and the corresponding nucleotide sequences. The proteins are predicted to be useful antigens for vaccines and/or diagnostics.

919 (46 kDa)

A) PURIFICATION



NEISSERIA MENINGITIDIS ANTIGENS AND COMPOSITIONS

This application is a continuation-in-part of the following U.S. Provisional Patent applications, from each of which priority is claimed, and each of which is incorporated by reference in its entirety: 60/083,758 (filed May 1, 1998); 60/094,869 (filed July 31, 1998); 60/098,994 (filed September 2, 1998); 60/099,062 (filed September 2, 1998); 60/103,749 (filed October 9, 1998); 60/103,794 (filed October 9, 1998); 60/103,796 (filed October 9, 1998); and 60/121,528 (filed February 25, 1999).

This invention relates to antigens from the bacterial species: *Neisseria meningitidis* and *Neisseria gonorrhoeae*.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

N. meningitidis causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the

United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants [*eg.* Morbidity and Mortality weekly report, Vol.46, No. RR-5 (1997)]. This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H.influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines*, *supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A and C. *Vaccine* 10:691-698).

Meningococcus B (menB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of α(2-8)-linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to menB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (*eg.* Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28).

Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (*eg.* Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonoccocal genes and proteins (*e.g.* EP-A-0467714, WO96/29412), but this is by no means complete. Other men B proteins may include those listed in WO 97/28273, WO 96/29412, WO 95/03413, US 5,439,808, and US 5,879,686.

The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae* including *Neisseria meningitidis* or *Neisseria gonorrhoeae*. Those sequences specific to *N. meningitidis* or *N. gonorrhoeae* that are more highly conserved are further preferred sequences.

It is thus an object of the invention is to provide Neisserial DNA sequences which encode proteins that are antigenic or immunogenic.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 20 shows an alignment comparison of amino acid sequences for ORF 235 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

Fig. 21 shows an alignment comparison of amino acid sequences for ORF 287 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

Fig. 22 shows an alignment comparison of amino acid sequences for ORF 519 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

Fig. 23 shows an alignment comparison of amino acid sequences for ORF 919 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

THE INVENTION

The invention provides proteins comprising the *N. meningitidis* amino acid sequences and *N. gonorrhoeae* amino acid sequences disclosed in the examples.

It also provides proteins comprising sequences homologous (i.e., those having sequence identity) to the *N. meningitidis* amino acid sequences disclosed in the examples. Depending on the particular sequence, the degree of homology (sequence identity) is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more). These proteins include mutants and allelic variants of the sequences disclosed in the examples. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between proteins is preferably determined by the Smith-Waterman

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2605>:

m760.seq

```
       1  ATGGGACAGT TTATGTCAGT TTTCCGCATC AATATGACCG CCGCCACGGT
      51  TTTGGCAGCA CTCTCGTCTT CGGTTTTTGC CGCACAAACG GAAGGTTTGG
     101  AAACCGTCCA TATTAAGGGT CAGCGTTCTT ACAACGCGAT TGCCACCGAG
     151  AAAAACGGCG ATTACAGCTC GTTTGCCGCC ACCGTCGGTA CAAAAATCCC
     201  CGCTTCTTTG CGCGAAATTC CGCAATCCGT CAGCATCATT ACCAACCAGC
     251  AGGTCAAAGA CCGCAATGTT GATACGTTTG ACCAGTTGGC ACGCAAAACG
     301  CCCGGCCTGC GCGTGTTGAG CAACGACGAC GGACGCTCTT CGGTTTACGC
     351  GCGCGGTTAC GAATACAGCG AATACAACAT CGACGGCCTG CCCGCGCAGA
     401  TGCAGAGTAT CAACGGCACG CTGCCCAACC TGTTCGCCTT CGACCGCGTG
     451  GAAGTGATGC GCGGGCCGAG CGGACTGTTC GACAGCAGCG GCGAGATGGG
     501  CGGCATCGTG AATCTGGTGC GCAAACGCCC GACCAAAGCG TTCCAAGGTC
     551  ATGCGGCGGC AGGGTTCGGT ACGCACAAAC AATATAAAGC CGAGGCGGAC
     601  GTATCGGGCA GCCTCAATTC AGACGGCAGC GTGCGCGGCC GCGTGATGGC
     651  GCAGACCGTC GGCGCGTCTC CGCGTCCCGC CGAGAAAAAC AACCGGCGCG
     701  AAACCTTCTA CGCGGCGGCG GATTGGGACA TCAACCCCGA TACGGTTTTG
     751  GGCGCGGGCT ATCTTTACCA GCAACGCCGC CTCGCGCCGT ACAACGGCCT
     801  GCCTGCCGAT GCCAATAACA AATTACCGTC CCTGCCGCAA CACGTATTTG
     851  TCGGCGCGGA TTGGAACAAA TTTAAAATGC ACAGCCACGA CGTGTTCGCC
     901  GATTTGAAAC ATTACTTCGG CAACGGCGGC TACGGCAAAG TCGGTATGCG
     951  CTATTCCGAT CGGAAAGCCG ATTCCAATTA TACGTTTGCG GGCAGCAAAC
    1001  TCAACAATAC CGGACAAGCC GACGTAGCGG GTTTGGGTAC GGACATTAAA
    1051  CAAAAAGCCT TTGCGGTTGA CGCAAGTTAC AGCCGTCCGT TTGCCTTGGG
    1101  CAACACCGCC AACGAATTTG TGATTGGTGC AGACTACAAC CGCTTGCGCA
    1151  GTACTAATGA ACAAGGGCGT TCGACTTTGT CAAAAAGCGT CGCTTTAGAT
    1201  GGTTTCCGCG CTTTGCCTTA TAACGGCATA CTTCAGAACG CCCGCGCCGG
    1251  AAACAAAGGT TTCAATCACT CCGTTACCGA AGAAAACCTC GACGAAACCG
    1301  GTTTGTATGC CAAGACGGTG TTCCGTCCTC TGGAAGGTTT GTCGTTGATT
    1351  GCAGGCGGAC GTGTAGGACA TCACAAAATC GAGTCGGGCG ACGGCAAAAC
    1401  CCTGCATAAA GCTTCGAAAA CCAAATTTAC AAGCTACGCC GGCGCGGTTT
    1451  ACGATATAGA CGGCAGCAAC AGCCTGTACG CTTCCGCCTC CCAACTCTAC
    1501  ACACCGCAAA CCAGCATCGG CACCGACGGC AAGCTGCTCA AACCGCGCGA
    1551  AGGCAACCAG TTTGAAATCG GCTACAAAGG CAGCTACATG GACGACCGCC
    1601  TCAATACCCG GGTTTCGTTC TACCGCATGA AGGATAAAAA CGCCGCCGCA
    1651  CCGCTGGACT CAAACAACAA AAAAACCCGT TACGCCGCAT TGGGCAAACG
    1701  CGTGATGGAA GGTGTTGAGA CCGAAATCAG CGGCGCGATG ACACCGAAAT
    1751  GGCAAATCCA TGCAGGTTAC AGCTACCTGC ACAGCCAAAT CAAAACCGCC
    1801  TCCAATTCGC GCGACGAAGG CATCTTCCTG CTGATGCCCA AACACAGCGC
    1851  AAACCTGTGG ACGACTTACC AAGTTACGTC CGGGCTGACC ATCGGCGGCG
    1901  GCGTGAACGC GATGAGCGGC ATTACTTCAT CTGCAGGGAT ACATGCAGGC
    1951  GGTTATGCCA CGTTCGATGC GATGGCGGCA TACCGCTTCA CGCCCAAACT
    2001  GAAGCTGCAA ATCAACGCCG ACAACATCTT CAACCGCCAT TACTACGCCC
    2051  GCGTCGGCAG CGAGAGCACC TTTAACATTC CCGGTTCGGA GCGCAGCCTG
    2101  ACGGCAAACC TGCGTTACAG TTTTTAA
```

This corresponds to the amino acid sequence <SEQ ID 2606; ORF 760>:

m760.pep

```
       1  MGQFMSVFRI NMTAATVLAA LSSSVFAAQT EGLETVHIKG QRSYNAIATE
      51  KNGDYSSFAA TVGTKIPASL REIPQSVSII TNQQVKDRNV DTFDQLARKT
     101  PGLRVLSNDD GRSSVYARGY EYSEYNIDGL PAQMQSINGT LPNLFAFDRV
     151  EVMRGPSGLF DSSGEMGGIV NLVRKRPTKA FQGHAAAGFG THKQYKAEAD
     201  VSGSLNSDGS VRGRVMAQTV GASPRPAEKN NRRETFYAAA DWDINPDTVL
     251  GAGYLYQQRR LAPYNGLPAD ANNKLPSLPQ HVFVGADWNK FKMHSHDVFA
     301  DLKHYFGNGG YGKVGMRYSD RKADSNYTFA GSKLNNTGQA DVAGLGTDIK
     351  QKAFAVDASY SRPFALGNTA NEFVIGADYN RLRSTNEQGR STLSKSVALD
     401  GFRALPYNGI LQNARAGNKG FNHSVTEENL DETGLYAKTV FRPLEGLSLI
     451  AGGRVGHHKI ESGDGKTLHK ASKTKFTSYA GAVYDIDGSN SLYASASQLY
     501  TPQTSIGTDG KLLKPREGNQ FEIGYKGSYM DDRLNTRVSF YRMKDKNAAA
     551  PLDSNNKKTR YAALGKRVME GVETEISGAM TPKWQIHAGY SYLHSQIKTA
     601  SNSRDEGIFL LMPKHSANLW TTYQVTSGLT IGGGVNAMSG ITSSAGIHAG
     651  GYATFDAMAA YRFTPKLKLQ INADNIFNRH YYARVGSEST FNIPGSERSL
     701  TANLRYSF*
```

CLAIMS

1. A protein comprising a fragment of an amino acid sequence from SEQ ID 2790 wherein said fragment comprises at least 7 amino acids from said sequence.

2. A protein comprising an amino acid sequence selected from the group consisting of even numbered SEQ IDs from SEQ ID number 2 through SEQ ID number 3020.

3. A protein having 50% or greater homology to a protein according to claim 1.

4. A protein comprising a fragment of an amino acid sequence selected from the group consisting of even numbered SEQ IDs from SEQ ID number 2 through SEQ ID number 3020, wherein said fragment comprises 7 or more consecutive amino acids from said sequence.

5. An antibody which binds to a protein according to any one of claims 1 to 3.

6. A nucleic acid molecule which encodes a protein according to any one of claims 1 to 3.

7. A nucleic acid molecule according to claim 5, comprising a nucleotide sequence selected from the group consisting of odd numbered SEQ IDs from SEQ ID number 1 through SEQ ID number 3019.

8. A nucleic acid molecule comprising a fragment of a nucleotide sequence selected from the group consisting of odd numbered SEQ IDs from SEQ ID number 1 through SEQ ID number 3019, wherein said fragment comprises 10 or more consecutive nucleotides from said sequence.

9. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to claim 5.

10. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to claim 6.

11. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to claim 7.

12. A composition comprising a protein, a nucleic acid molecule, or an antibody according to any preceding claim.

13. A composition according to claim 11 being a vaccine composition or a diagnostic composition.

14. A composition according to claim 11 for use as a pharmaceutical.

15. The use of a composition according to claim 11 in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria.

16. A composition comprising a protein of claim 1 wherein said composition is immunogenic.

17. A composition comprising a protein of claim 2 wherein said composition is immunogenic.

18. A composition comprising a protein of claim 3 wherein said composition is immunogenic.